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Biochemical Bone Turnover Markers: Significance in Patients with Osteoporosis

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A B S T R A C T

Osteoporosis is a systemic disease, which is characterized by reduced bone mass and microarchitectural deterioration of the bone tissue, resulting in an increased risk of fracture. Since osteoporosis is today a disease with high incidence rate, the aim of this study was to determine a correlation between bone mass density (BMD) and concentration of biochemical bone turnover markers – deoxypyridinoline (DPD) as a marker of bone resorption, and osteocalcin (OC) as a marker of bone formation. The study included 70 women between 33 and 76 years of age. In all women BMD was measured by Dual X-ray Absorptiometry (DXA) as a T-score. T-score was defined as the number of standard deviations of the bone mass density from the maximum bone mass density in young adults. According to T-score, patients were divided into three groups: patients with osteoporosis, patients with osteopenia and control group consisting of patients with normal T-score. DPD in urine and OC in serum were measured by a routine procedure. Results: a negative correlation between BMD and concentration of bone turnover marker was discovered. One-way analysis of variance and Pearson correlation were used for statistical analysis, with a P value <0.05 being considered significant. Although a negative correlation was discovered, we concluded that both procedures have a significant role in diagnosis and follow-up of patients with osteoporosis.

Key words: *osteoporosis, bone mass density, osteocalcin, deoxypyridinoline*

Introduction

Osteoporosis is a systemic disease, which is characterized by a low bone mass and microarchitectural deterioration of the bone tissue, resulting in an increased risk of fracture[1]. Remodeling processes are conducted in bone tissue during a lifetime, i.e. bone resorption and bone formation processes, which are in balance in healthy tissue. A prolonged imbalance in bone metabolism between bone resorption and formation, with bone resorption exceeding formation, may result in increased bone fragility[2,3]. Bone Mass Density (BMD) is measured by densitometry, most commonly by Dual X-ray Absorptiometry (DXA). The most valuable parameter in DXA is T-score. T-score is defined as the number of standard deviations of the bone mass density from the maximum bone mass density in young adults. World Health Organization (WHO) has defined diagnostic criteria for osteoporosis based on T-score where T-score between –1.0 and –2.5 is defined as osteopenia and T-score below –2.5 is defined as osteoporosis[4].

Bone formation and resorption data can be achieved by measuring biochemical markers of bone turnover (BTMs) in serum and urine. According to the literature search, BTMs may be useful as: screening method, diagnostic criteria, assessment of the stage of disease or risk factors assessment, and documentation of effects of therapeutic agents or determination of therapy initiation[5]. By assessing the diagnostic value of BTMs in female patients with suspected osteoporosis, we have studied correlation between the BMD and concentrations of deoxypyridinoline (DPD) and osteocalcin (OC).

We have measured concentrations of two biochemical bone turnover markers, DPD as a marker of bone resorption and OC as a marker of bone formation and compared them to the BMD results. Osteocalcin is considered to be a specific marker for osteoblast function and is the main noncolagen protein of the bone matrix. Osteocalcin is synthesized by osteoblasts, odontoblasts, and

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hypertrophic chondrocytes. After synthesis, which is significantly stimulated by calcitriol, it binds to hydroxyapatite, and much of it is deposited in the bone matrix. A smaller component of newly synthesized osteocalcin fragments are released from the bone matrix into circulation where its concentration can be measured by assays for circulating osteocalcin[6,7].

Pyridinoline (PYD; hydroxylysylpyridinoline) and deoxypyridinoline (DPD; lysylpyridinoline) are two non-reduced, cross-linked molecules of pyridine in mature bone collagen. By resorption of the collagen, DPD molecules are secreted into circulation and excreted in urine, either as free or as peptide bound moieties[6,7].

Materials and Methods

Study population

The study included 70 women between 33 and 76 years of age with suspected osteoporosis. Patients were included in the study on their initial visit to the Clinical Department of Nuclear Medicine and Radiation Protection – Reference Centre of the Ministry of Health and Social Welfare of the Republic of Croatia, Division for Diagnosing Parathyroid Gland Diseases. Concentration of DPD in urine and OC in serum was measured in all women.

Urine and blood sampling and analysis

Second morning urine sample was collected and DPD in urine was measured. Sample was collected in the morning after a patient urinated first at home and then again in the hospital around 8 am into a special canister. Urine samples were divided into 1 mL aliquots and saved for competitive enzyme immunoanalisys of DPD by commercially available kit, according to Manufacturer's protocol (Metra Biosystem INC., Mountain View, SAD).

Reference values of DPD for women: 3.0–7.4 nM DPD/mM creatinine and for men: 2.3–5.4 nM DPD/mM creatinine. OC was measured in a serum of patients after an overnight fasting, drinking only water. 8 mL of vein blood was collected and stored immediately on ice to prevent sample degradation. After 15 to 30 minutes sample was coagulated, centrifuged and analyzed by immunoradiometric method (IRMA) with commercially available kits, according to Manufacturer's recommendations (BioSource Europe S.A., Nivelles, Belgium, reference value of OC: 5–25 ng/mL).

Densitometry analysis

Bone Mass Density (BMD) was measured by Dual X-ray Absorptiometry (DXA) at the Department of Endocrinology in the scope of the Clinic for Internal Diseases at the University Hospital Centre Osijek.

Statistical analysis

One-way analysis of variance and Pearson correlation were used for statistical analysis, with a p value <0.05 being considered significant.

Results

According to T-score, patients were divided into three groups: patients with osteoporosis, patients with osteopenia and control group consisting of patients with normal T-score. Osteopenia (Table 1) was discovered in 44.28% of patients. In this group DPD was normal in 45.17% and increased in 54.83% of patients. OC concentrations were normal in 80.65%, increased in 6.45% and decreased in 12.9% of patients with osteopenia. Osteoporosis (Table 2) was discovered in 32.85% of patients. In patients with osteoporosis DPD was normal in 39.14 % of patients, increased in 56.52% and decreased in 4.34% of patients. In the same group of patients, OC was normal in 91.13 % and decreased in 8.69% of patients. Control

TABLE 1
PATIENTS WITH OSTEOPENIA

Osteopenia				
Number of pts.	Age/years	DPD (nM/mM creatinine)	Osteocalcin (ng/mL)	T-score
1	64	5.41	10.60	–1.9
2	65	4.30	7.94	–1.3
3	68	11.18	5.02	–1.3
4	54	10.85	21.3	–2.0
5	65	6.42	4.96	–2.3
6	71	5.31	9.25	–2.3
7	68	12.35	7.10	–2.1
8	63	12.36	5.87	–1.9
9	63	10.14	16.30	–2.3
10	58	3.09	1.30	–2.4
11	58	13.42	25.40	–1.3
12	73	6.50	4.26	–1.6
13	54	13.59	16.50	–2.4
14	70	13.48	11.60	–1.6
15	48	10.31	16.20	–2.3
16	57	5.06	8.60	–2.0
17	67	6.57	5.16	–2.3
18	69	5.51	27.90	–1.7
19	73	10.33	4.21	–1.9
20	33	6.17	5.72	–1.0
21	59	4.10	6.77	–1.9
22	48	12.86	8.51	–1.6
23	64	6.08	9.57	–2.0
24	72	6.18	11.90	–2.3
25	73	5.38	13.30	–1.5
26	76	12.26	14.70	–2.3
27	65	10.40	17.90	–1.6
28	58	12.80	19.90	–1.9
29	52	13.01	11.90	–2.1
30	53	8.36	9.16	–2.4
31	59	8.19	19.10	–2.0

TABLE 2
PATIENTS WITH OSTEOPOROSIS

Osteoporosis				
Number of pts.	Age/years	DPD (nM/mM creatinine)	Osteocalcin (ng/mL)	T-score
1	65	5.34	14.80	–2.7
2	73	1.65	1.95	–3.4
3	56	9.94	12.00	–2.5
4	64	18.05	14.80	–3.3
5	53	4.91	11.60	–3.2
6	72	11.25	18.90	–4.8
7	70	11.36	19.40	–3.3
8	59	5.84	6.86	–2.8
9	58	12.16	16.40	–3.5
10	54	13.28	13.00	–3.8
11	75	9.03	12.20	–3.0
12	69	12.97	14.70	–4.5
13	68	10.03	21.30	–2.7
14	59	6.04	8.01	–2.6
15	73	4.54	7.56	–3.3
16	51	11.54	13.80	–3.8
17	55	12.99	13.40	–2.8
18	70	4.90	8.80	–3.0
19	75	3.84	7.95	–4.3
20	67	9.71	14.40	–3.5
21	54	7.33	11.20	–3.0
22	62	5.17	5.64	–2.7
23	67	8.94	2.54	–3.0

TABLE 3
CONTROL GROUP OF EXAMINEES

Normal				
Number of pts.	Age/years	DPD (nM/mM creatinine)	Osteocalcin (ng/mL)	T-score
1	57	6.93	10.90	0.4
2	58	4.75	7.31	2.8
3	37	5.71	5.97	–0.1
4	51	5.71	11.20	2.1
5	41	4.26	6.41	0.7
6	55	6.02	12.00	0.2
7	71	6.00	5.78	1.0
8	54	14.40	15.80	0.0
9	63	8.09	19.30	0.0
10	71	9.83	4.36	0.4
11	57	8.05	5.13	2.4
12	59	12.65	12.50	–0.6
13	51	10.32	7.43	–0.6
14	54	8.54	15.00	0.2
15	57	16.79	12.80	0.1
16	73	14.40	14.50	0.0

group (Table 3) consisted of 22.85% of patients. DPD was normal in 47.75% and increased in 56.25% of the control group. OC was normal in 93.75% of the control group, whereas it was decreased in only 6.25%. All patients have signed consent on being informed before being included in the study.

Discussion and Conclusion

This study has shown a negative correlation between biochemical markers of the bone turnover and BMD. Negative correlation has been established for DPD, as well as for OC. DPD was increased in about a half of the patients, whereas OC values were in the range of referential values in patients with osteoporosis, as well as in patients with osteopenia. Data obtained in this study are consistent with previous reports where the relationship between markers and BMD was either not established, or where a very weak correlation was established[8–11]. These studies analyzed a larger number of biochemical markers of the bone turnover. However, there are also different data published, in which a positive correlation between biochemical markers of the bone turnover and BMD has been established[12]. Although data published in different studies are contradictory, biochemical markers of the bone turnover still play an important role in diagnosis, treatment and follow-up of osteoporosis. It has also been established that levels of biochemical markers of the bone turnover are important factor for identifying the response of the bone tissue after starting with the therapy[13,14]. Also, it is known that reduced levels of resorption markers can occur even two weeks after the beginning of bisphosphonate therapy and concentration plateau can be reached after 3 to 6 months. Reduced levels of bone formation markers occur somewhat later[9,10]. Moreover, bone turnover markers can be a useful tool in predicting a risk of future osteoporotic fractures[14–20], what is very important for women excluded from the fragile bone risk group based on DXA results. In general, measurement of biochemical bone turnover markers has its advantages and disadvantages. Advantages include identification of the rapid bone loss, weekly evaluation of responses to therapy, as well as monitoring the results of the therapy. Disadvantages include lack of consensus in regard to constitution of high and low risk groups for fracture occurrence, poor relationship between BMD measurements and concentration of bone turnover markers and expenses of measuring bone turnover markers, which could also be a limiting factor. Also, disadvantages could be variations in measurement of bone resorption markers, because their concentrations are affected by age, menopause, occurrence of previous fractures and bone size[21–28]. One of the limiting factors in detection of bone turnover marker concentration is also inability to asses a total bone loss and to predict future bone loss[9]. However, regarding all these issues and because of the high inci-

dence rate of osteoporosis in general population[29–34] and associated bone fracture risk, future plan is to conduct a similar study with larger number of participants included in the study and to measure a larger number of biochemical bone turnover markers.

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DIJAGNOSTIČKA VRIJEDNOST BIOKEMIJSKIH MARKERA KOŠTANE PREGRADNJE U BOLESNICA SA SUMNJOM NA OSTEOPOROZU

SAŽETAK

Osteoporoza je sistemska bolest koju karakterizira smanjenje koštane mase i propadanje mikroarhitekture koštanog tkiva, a posljedica je povećan rizik od nastanka fraktura. Kako je osteoporoza danas vrlo raširena bolest, svrha istraživanja bila je ustanoviti korelaciju između gustoće koštane mase (BMD – Bone Mass Density) i koncentracija biokemijskih markera koštane pregradnje – deoksipiridinolina (DPD) kao markera koštane razgradnje i osteokalcina (OC) kao markera formiranja kosti. Istraživanje je provedeno u 70 žena dobi između 33 i 76 godina. U svih žena denzitometrijskim mjerenjem (DXA – Dual X-ray Absorptiometry) BMD izražena je s T-vrijednošću. T-vrijednost je broj standardnih odstupanja koštane gustoće od maksimalne koštane mase mladih odraslih osoba. S obzirom na T-vrijednost, bolesnice su podijeljene u dvije skupine: bolesnice s osteopenijom i bolesnice s osteoporozom. Kontrolnu skupinu činile su ispitanice s urednom T-vrijednosti. Određene su vrijednosti biokemijskih markera koštane pregradnje, DPD-a u urinu i OC-a u serumu. Rezultati istraživanja ukazuju na negativnu korelaciju između BMD-a i koncentracija navedenih biokemijskih markera koštane pregradnje. U stastističkoj analizi korišteni su jednostrana analiza verijance i Pearsonov korelacijski test s pragom signifikantnosti $p<0,05$. Unatoč dobivenoj negativnoj korelaciji između DPD-a i OC-a i BMD-a, mišljenja smo da određivanja koncentracija markera koštane razgradnje i formiranja kosti imaju značajnu ulogu u dijagnostici i praćenju bolesnika s osteoporozom.